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(54) Chemical capping by phosphitylation during oligonucleotide synthesis

Chemische Blockierung durch Phosphithylierung während der Oligonukleotid-Synthese

Blocage chimique par phosphitylation au cours de la synthèse d'oligonucléotides

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EP-A- 0 219 342

- **TETRAHEDRON LETTERS, vol. 27, no. 4, 1986, pages 469-472, Pergamon Press Ltd, Oxford, GB; B.C. FROEHLER et al.: "Nucleoside H-phosphonates: Valuable intermediates in the synthesis of deoxyoligonucleotides"**
- **SYNTHESIS, no. 5, May 1984, pages 410-413; D.E. GIBBS et al.: "Bis[2,2,2- trifluoroethyl] phosphite, a new reagent for synthesizing mono- and diesters of phosphorous acid**

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Description

The invention relates generally to methods for synthesizing oligonucleotides, and more particularly, to the use of phosphite monoesters to chemically cap failure sequences in either DNA or RNA synthesis.

Genes and gene control regions can now be routinely characterized and studied at the molecular level. This has been made possible by recent advances in the technology associated with analyzing, modifying, and synthesizing DNA and RNA. Of particular importance has been the development of machines for the automated synthesis of support-bound single stranded DNA, e.g. Matteucci and Caruthers, J. Amer. Chem. Soc., Vol. 103, pgs. 3185-3191 (1981); and Gait, ed., Oligonucleotide Synthesis: A Practical Approach (IRL Press, Washington, D.C., 1984).

The methods of choice for conducting automated DNA synthesis are the phosphoramidite and hydrogen-phosphonate chemistries, e.g. Beaucage and Caruthers,Tetrahedron Letters Vol. 22, pgs. 1859-1862 (1981); McBride and Caruthers, Tetrahedron Letters, pgs. 245-248 (1983); Froehler and Matteucci, Tetrahedron Letters, Vol. 27, pgs. 469-472 (1986); Garegg et al, Tetrahedron Letters, Vol. 27, pgs. 4051-4054 (DNA synthesis) and pgs. 4055-4058 (RNA synthesis)(1986); and Froehler et al, Nucleic Acids Research, Vol. 14, pgs. 5399-5407 (1986). A synthetic cycle is repeated under computer control to add one nucleoside monomer unit at a time to achieve the desired sequence and length which defines the oligonucleotide. For example, within the phosphoramidite, or phosphite triester, synthetic cycle several reactions are necessary:

I. Deprotect the reactive functionality (usually a 5′ hydroxyl) on the growing chain;

II. Achieve coupling by the addition of a monomer and activator;

III. Cap unreacted 5′ hydroxyls to prevent further coupling to failure sequences; and

IV. Oxidize the newly formed internucleotide phosphorous linkage to the naturally occurring pentacoordinate state.

The phosphoramidite method is highly optimized, allowing the construction of oligonucleotides as much as 175 nucleotides in length, Efcavitch, S.W., pgs. 65-70 in Biophosphates and Their Analogues: Synthesis, Structure, Metabolism, and Activity, Bruzik and Stec, eds. (Elsevier, Amsterdam, 1987). Such performance requires an average yield per cycle of greater than 99%. An essential feature of the synthesis cycle is an effective capping reaction to permanently remove unreacted growing chains from participation in subsequent cycles. Without capping, failure sequences or deletion sequences, those oligonucleotides missing one or more monomeric nucleotides with respect to the desired sequence, will attain a greater average length than they would with capping. The utility of capping is to minimize the length and presence of failure sequences. With capping, a higher concentration of monomeric nucleotide is available to the correctly growing sequences of DNA. Moreover, with an efficient capping reaction performed each cycle, the correct sequence DNA, or product, is more easily located, and thus purified by conventional means, such as gel electrophoresis of HPLC. The presence of failure sequences having nearly identical size and composition as the product makes purification extremely difficult.

During phosphoramidite DNA synthesis, failure sequences are capped by acetylation, effected by the concurrent delivery of acetic anhydride and dimethylaminopyridine (DMAP) to the synthesis column. The resulting 5′ acetate ester cap prevents the sequence of DNA from participating in subsequent condensation reactions in the synthesis. Unfortunately, however, the acetate ester cap is removed during the post-synthesis ammonia cleavage/deprotection step, which makes failure sequence contaminants available to participate in a variety of enzymatic reactions for which the complete sequences were prepared. Such participation, for example, could measurably reduce the efficiency by which DNA linkers are constructed making their use in recombinant vectors more difficult. The availability of a cap which survived the post-synthesis cleavage/deprotection step would be highly useful.

For the hydrogen-phosphonate method, capping by acetylation is not possible. Acetylation capping of the unreacted 5′ hydroxyls of failure sequences occurs at a useful rate only by catalysis with a strong base, such as DMAP, N-methylimidazole, or triethylamine. The internucleotide hydrogen phosphonate linkage is modified by phosphorous acetylation under the influence of these strong bases. The phosphorous acetylated residues are then susceptible to cleavage during the post-synthesis cleavage/deprotection step, resulting in internucleotide scission.

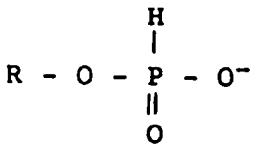
It has been claimed that a discrete capping step is unnecessary in the hydrogen-phosphonate method due to acylation of unreacted 5′hydroxyls of failure sequences during the condensation step, e.g. Froehler and Matteucci (cited above) and Froehler et al (cited above). Acylation can occur by esterification of 5′ hydroxyls by the commonly used acid chloride activators or by the reactive coupling intermediate. The acid chloride activator is present during the coupling reaction to form the reactive coupling intermediate with monomers. However, it has been demonstrated that coupling and acylation can be incomplete during the condensation step, leaving a certain amount of 5′ hydroxyl available for increasing the size of failure sequences during subsequent cycles of synthesis. An effective capping operation for hy-

drogen-phosphonate DNA synthesis is clearly desirable.

SUMMARY OF THE INVENTION

The invention is a method of capping failure sequences in oligonucleotide synthesis by phosphitylation. Preferably, the method involves solid phase, or support-bound, oligonucleotide synthesis by phosphoramidite, phosphotriester, and/or nucleoside hydrogen phosphonate chemistries. Capping is achieved by reacting a phosphite monoester capping agent with the 5′ or 3′ hydroxyl of the failure sequences between successive condensation steps in the synthesis procedure. The 3′ or 5′ phosphite diester substituent of the failure sequence is inert with respect to subsequent reaction steps in the synthesis of the desired oligonucleotide product.

As used herein, the term capping refers to reacting either the free 5′ hydroxyl of a 3′ to 5′ growing nucleotide chain or the free 3′ hydroxyl of a 5′ to 3′ growing nucleotide chain with a capping agent to render the chain incapable of participating in subsequent condensation steps. The preferred capping agents of the invention are phosphite monoesters of the form:



Formula I

wherein R, either alone or together with the oxygen to which it is attached, is unreactive with the reagents used in solid phase oligonucleotide synthesis, particularly phosphoramidites or nucleoside hydrogen phosphonates. Preferably, R represents a lower alkyl, an electron-withdrawing substituted lower alkyl, a lower alkyl- or halo-substituted aryl, or a heterocycle containing nitrogen, oxygen, or sulfur and from 5-8 carbon atoms. More preferably, R is methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl, cyclopentylmethyl, isopentyl, neopentyl, n-hexyl, neohexyl, isohexyl, cyclohexylmethyl,beta-cyclopentylethyl, lower alkyl- or halo-substituted phenyl, lower alkyl- or halo-substituted benzyl, or lower alkyl- or halo-substituted phenylethyl, morpholinyl, thiomorpholinyl, piperidinyl, piperazinyl, beta-electron-withdrawing-substituted ethyl, or the like. In further preference, the electron-withdrawing substituent of beta-electron-withdrawing-substituted ethyl is cyano, nitro, phenylsulphonyl, or phenylester. Most preferably, the beta-electron-withdrawing-substituted ethyl is beta- cyanoethyl. In further preference, the lower alkyl- or halo-substituents of the lower alkyl- or halo-substituted phenyl and benzyl are methlyl, chloro, or bromo. In further preference, morpholinyl, thiomorpholinyl, and piperidinyl are morpholino, thiomorpholino, and piperidino, respectively.

As used herein, the term lower alkyl refers to straight-chained, branched, or cyclic alkyls containing from 1 to 6 carbon atoms.

"Electron-withdrawing" denotes the tendency of a substituent to attract valence electrons of the molecule of which it is apart, i.e. it is electronegative, March, Advanced Organic Chemistry, pgs. 16-18 (John Wiley, New York, 1985).

As used herein, the term oligonucleotide refers to a single stranded chain of either deoxyribonucleotides or ribonucleotides having from a few, e.g. 2-20, to many, e.g. 20 to several hundred or more, nucleotides.

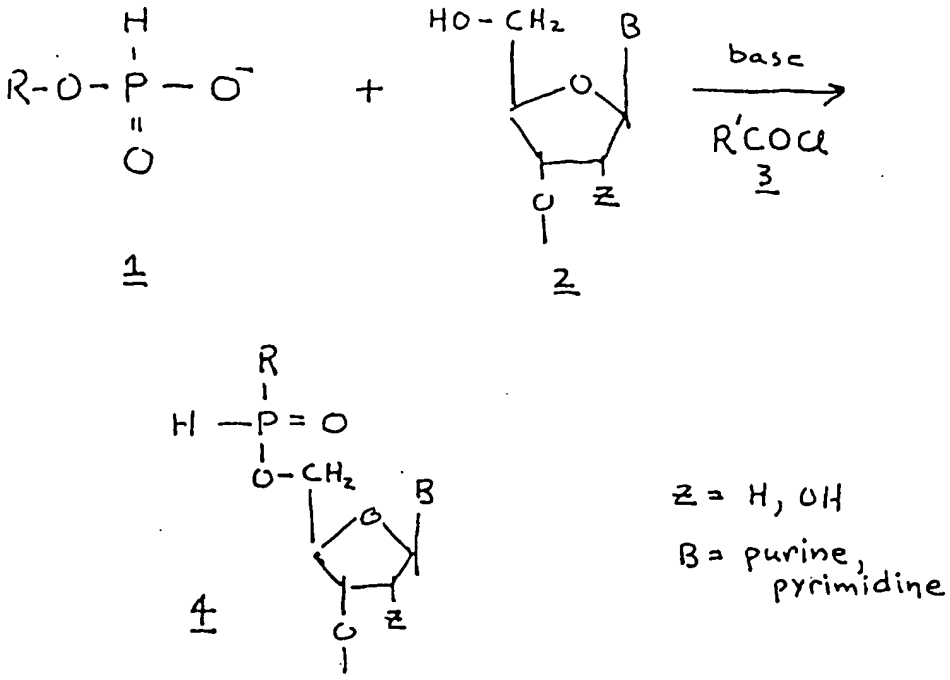
The chemical structures illustrated by Formula I are referred to in the literature as both phosphites and phosphonates. Reflecting the approximate usage in the literature, throughout the structures will be referred to as phosphites, except when R is a nucleoside. In such cases the structure will be referred to as a hydrogen or H- phosphonate.

The present invention overcomes deficiencies in both the nucleoside hydrogen phosphonate and the phosphite triester methods of oligonucleotide synthesis. Use of the capping step in the nucleoside hydrogen phosphonate synthesis process significantly enhances yields by reducing the average length of failure sequences. In both the nucleoside hydrogen phosphonate method and the phosphite triester method, attachement of the capping agents of the invention renders the failure sequences incapable of participating in subsequent biological experiments for which the complete-sequence products are destined, e.g. 5′ enzymatic phosphorylation, either for labeling with ^{32}P, or as a pretreatment for subsequent ligation to other pieces of DNA.

Figure 1 presents data illustrating the relative purity of reaction products from hydrogen phosphonate syntheses of 34-mer oligonucleotides without (lane 1) and with (lane 2) the capping step of the invention.

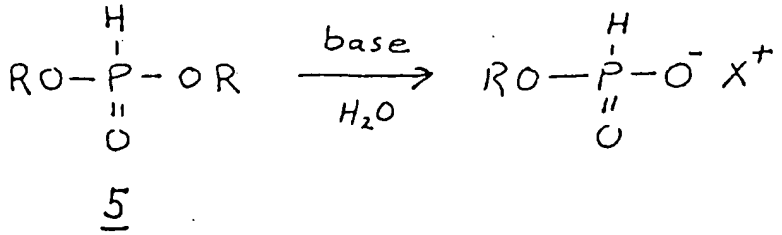
The invention includes a method for capping failure sequences in oligonucleotide synthesis, and methods of syn-

thesizing oligonucleotides which include the capping method of the invention as a step. As illustrated by Formula II, the capping method of the invention comprises reacting a phosphite monoester defined by Formula I, 1, with the free 5′ or 3′ hydroxyl of a failure sequence, 2, in the presence of a sterically hindered acid chloride, 3, to form a phosphite diester, 4, between the failure sequence and a group which is inert to subsequent reaction steps.



Formula II

Preferably, the capping agents of the invention (1 in Formula III below) are prepared by alkaline hydrolysis of the symmetrical phosphite diesters, 5, as described by Gibbs et al in Synthesis, pgs. 410-413 (1984), which is incorporated by reference. The phosphite monoester 1 can be used directly as a salt after evaporating volatile by products of the reaction or after purification by conventional means.



Formula III

In the sterically hindered acid chloride 3, R′ is preferably tert-butyl, sec-butyl, cyclohexyl, adamantyl, norbornyl, phenyl, aryl, or the like. More preferably, R′ is tert-butyl, norbornyl, or adamantyl. Most preferably, R′ is adamantyl.

Preferably, X^+ is ammonium, lower alkylammonium, pyridinium, lutidinium, cyclohexylammonium, a metal salt cation such as Na^+, K^+, Li^+, Ba^+, Mg^+, or the like. More preferably, X^+ is triethylammonium, tetrabutylammonium, diisopropylethylammonium, pyridinium, lutidinium, or cyclohexylammonium. Most preferably, X^+ is triethylammonium, tetrabutylammonium, or diisopropylammonium.

Preferably, prior to delivery to the synthesis column bearing the oligonucleotide, a phosphite monoester of the invention and its cationic counter ion are dissolved in a solution comprising an aprotic polar solvent, such as acetonitrile, tetrahydrofuran, dichloromethane, or the like, or some combination thereof, and a mild base such as pyridine, picoline,

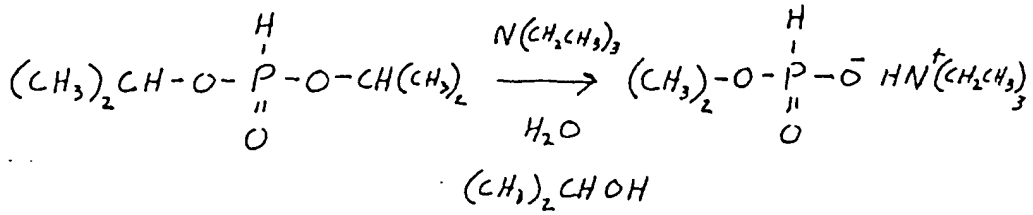
lutadine, collidine, or the like. Pyridine is the most preferred mild base. Preferably, the concentration of the phosphite monoester is between about 0.1 to 1.0 molar. Likewise, the sterically hindered acid chloride (3 in Formula II), prior to delivery to the synthesis column, is dissolved in a solution comprising an aprotic polar solvent, such as acetonitrile, tetrahydrofuran, dichloromethane, or the like, or some combination thereof, and a mild base such as pyridine, picoline, lutadine, collidine, or the like. Pyridine is the most preferred mild base. The respective solutions are delivered concurrently to the synthesis column bearing the growing oligonucleotide so that approximately equimolar amounts of the phosphite monoester and sterically hindered acid chloride are present in the reaction mixture. This operation can be readily performed by an automated DNA synthesizer, such as the Applied Biosystems models 380A, 380B, or 381A. The capping procedure of the invention is performed as a step in each cycle, after the coupling reaction, to render the failure sequences inert. Preferably, the synthesis column is immersed in the reaction mixture for about 20-120 seconds at room temperature, after which the reagents are flushed from the column with a solvent, such as acetonitrile, tetrahydrofuran, dichloromethane, pyridine, or the like, or some combination thereof. All vessels within the instrument must be maintained rigorously free of moisture and oxygen under an atmosphere of an inert gas, such as argon.

Detailed procedures for the phosphite triester and hydrogen phosphonate methods of oligonucleotide synthesis are described in the following references, which are incorporated by reference: Caruthers et al, U.S. Patents 4,458,066 and 4,500,707; Matteucci et al, J. Amer. Chem. Soc., Vol. 103, pgs. 3185-3191 (1981); Caruthers et al, Genetic Engineering, Vol. 4, pgs. 1-17 (198); Jones, chapter 2, and Atkinson et al, chapter 3, in Gait, ed., Oligonucleotide Synthesis: A Practical Approach (IRL Press, Washington, D.C., 1984); Froehler et al, Tetrahedron Letters, Vol. 27, Pgs. 469-472 (1986); Garegg et al, Tetrahedron Letters, Vol. 27, pgs. 4051-4054 and 4055-4058 (1986); and Froehler et al, Nucleic Acids Research, Vol. 14, pgs. 5399-5407 (1986).

The following examples illustrate the invention.

EXAMPLES

Example I. Synthesis of Isopropylphosphite Triethylammonium Salt



Diisopropylphosphite (10.0 g, 0.06 moles), triethylamine (14.6 g, 0.14 moles), isopropanol (20 ml), and water (10 ml) were mixed in a flask under an argon atmosphere and heated at 60°C for 48 hours. The volatile components were removed under vacuum, leaving a viscous, clear oil. The resulting product was produced in 95% yield (12.8 g) and had the following spectral data:

^{1}H nmr (acetone d6, chemical shifts relative to TMS): 10.1 + 3.3 (d, 1H, J=610 Hz), 4.4 (m, 1H), 3.15 (q, 6H, J=7 Hz), 1.35 (d, 6H, J=7 Hz), 1.20 (t, 9H, J=7 Hz)

^{31}P nmr (acetone d6, chemical shift relative to H_3PO_4): 1.10 ppm J=610 Hz

Example II. Synthesis of Ethylphosphite Triethylammonium Salt

The triethylammonium salt of ethylphosphite was synthesized by the same procedure as Example I to give a product having the following spectral data:

^{1}H nmr (acetone d6): 10.0 + 3.4 (d, 1H, J=599 Hz), 3.85 (q, 2H, J=7 Hz), 3.15 (q, 6H, J=7 Hz), 1.32 (t, 9H, J=9 Hz), 1.20 (t, 3H, J=7 Hz)

^{31}P nmr (acetone d6): 0.64 ppm J=599 Hz

Example III. Reaction of Triathylammonium isopropyl phosphite with Thymidine Attached to a Solid Support

A solution consisting of 0.1 M triethylammonium isopropyl phosphite in 1:1 acetonitrile:pyridine, and a solution con-

sisting of 0.1 M 1-adamantane carboxylic acid chloride in 1:1 acetonitrile:pyridine were delivered concurrently to 1.0 micromole of thymidine linked via a 3' succinate to a controlled-pore glass support in an Applied Biosystems model 380B DNA synthesizer. After approximately 30 seconds, the solutions were removed from the column, and the column was washed with acetonitrile. After oxidation of phosphite diester linkage, the product was cleaved from the support with ammonia and subjected to HPLC analysis. By comparison with an authentic sample, it was determined that the major component of the product was 5'-isopropylphosphate thymidine.

Example IV. Synthesis of a 34 Base Oligonucleotide by the Hydrogen Phosphonate Method With and Without Capping

The same 34-mer oligonucleotide, 5'-AGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTAT, was twice synthesized by the hydrogen phosphonate method on an Applied biosystems model 380B DNA synthesizer following the procedure described by Froehler et al (cited above), once including the capping step of the invention, and once excluding the capping step. The capping step was performed using the reagents and reaction conditions of Example III.

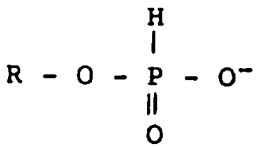
Figure 1 illustrates the results of the gel electrophoretic separation of the material cleaved from the respective columns: lane 1 contains the material produced without capping, and lane 2 contains the material produced with capping. The material in both lanes was visualized by UV shadowing. It can be readily seen that the material in lane 2 contains fewer failure sequences near the 34-mer product, as determined by the intensity of lower molecular weight bands near the 34-mer on the gel.

Example V. Synthesis of an 18 Base Oligonucleotide by the Hydrogen Phosphonate Method With and Without Capping

The 18-mer oligonucleotide, 5'-TCACAGTCTGATCTCGAT, was synthesized twice by the hydrogen phosphonate method, once with capping and once without capping, following the same procedure as Example IV. The material cleaved from each column was analyzed by HPLC and the ratio of the correct sequence product to the most prevalent class of failure sequences (17-mers) was determined from the areas under the respective peaks on the chromatograms. The ratio with capping was 33.9. The ratio without capping was 4.9.

Claims

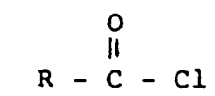
1. A method of capping failure sequences in solid phase oligonucleotide synthesis, comprising condensing a capping agent with a hydroxyl of a failure sequence, the capping agent being defined by the formula:



wherein R is lower alkyl, electron withdrawing-substituted lower alkyl, lower alkyl-substituted or halo-substituted aryl, or a nitrogen-, oxygen- or sulfur- containing heterocycle having from 5 to 8 carbon atoms.

2. A method according to claim 1 wherein the capping agent is condensed with the hydroxyl in the presence of a sterically hindered acid chloride.

3. A method according to claim 2, wherein R is a straight-chained, branched or cyclic alkyl having from 1 to 6 carbon atoms, morpholinyl, thiomorpholinyl, piperidinyl, piperazinyl or a beta-electron-withdrawing substituted ethyl, and wherein the sterically hindered acid chloride is defined by the formula:

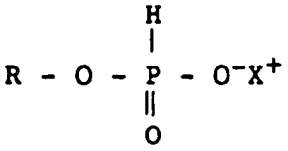


wherein R' is tert-butyl, sec-butyl, cyclohexyl, adamantyl, norbornyl or phenyl.

4. A method according to any one of claims 1 to 3, wherein R is a straight-chained or branched alkyl of 1 to 4 carbon atoms, phenylethyl, beta-cyanoethyl, morpholino, piperidino, thiomorpholino, beta-nitroethyl, beta-lower alkyl-sub-

stituted sulphonyl- or beta-phenyl-substituted ethyl, lower alkyl-substituted or halo-substituted-phenyl or benzyl.

5. A method according to any one of claims 1 to 4, wherein the capping agent is a salt defined by the formula:



wherein X^+ is ammonium, lower alkylammonium, pyridinium, lutidinium, cyclohexylammonium or a metal salt cation.

6. A method according to claim 5, wherein X^+ is triethylammonium, diisopropylammonium, tetrabutylammonium, diisopropylethylammonium, pyridinium, lutidinium or cyclohexylammonium.

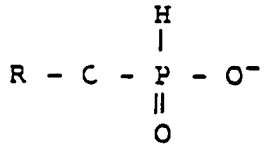
7. A method according to any one of claim 1 to 6, wherein the sterically hindered acid chloride and the capping agent are present in equimolar amounts.

8. A method of synthesizing an oligonucleotide of a predetermined sequence on a solid support, the method comprising the steps of:

(a) deprotecting a 5'-protected oligonucleotide attached to the solid support to form a deprotected oligonucleotide;
(b) reacting a 5'-protected nucleotide monomer with the deprotected oligonucleotide to form either a 5'-protected oligonucleotide or a failure sequence, the failure sequence having a 5' hydroxyl;
(c) capping the failure sequence by reacting a capping agent with the 5' hydroxyl of the failure sequence by a method according to any one of claims 1 to 7; and
(d) repeating steps (a), (b) and (c) until the oligonucleotide of the predetermined sequence is obtained.

Patentansprüche

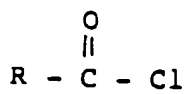
1. Ein Verfahren zum Blockieren von Fehlsequenzen in der Festphasen-Oligonucleotidsynthese, aufweisend das Kondensieren eines Blockierungsmittels mit einer Hydroxylgruppe, einer Fehlsequenz, wobei das Blockiermittel durch die Formel definiert worden ist:



wobei R ein niederes Alkyl, ein elektronenabstoßendes substituiertes niederes Alkyl, ein niederes alkylsubstituiertes oder halosubstituiertes Aryl oder ein Stickstoff, Sauerstoff oder Schwefel enthaltender Heterozyklus mit 5 bis 10 Kohlenstoffatomen ist.

2. Ein Verfahren nach Anspruch 1, wobei das Blockiermittel mit der Hydroxylgruppe in Anwesenheit eines sterisch gehinderten Säurechlorids kondensiert wird.

3. Ein Verfahren nach Anspruch 2, wobei R ein geradkettiges, verzweigtes oder zyklisches Alkyl mit 1 bis 6 Kohlenstoffatomen, Morpholinyl, Thiomorpholinyl, Piperidinyl, Piperazinyl oder ein beta-elektronenabstoßendes substituiertes Ethyl ist und wobei das sterisch gehinderte Säurechlorid durch die Formel definiert ist:

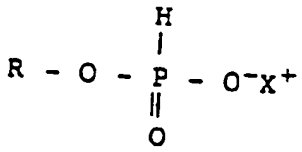


wobei R' tert. Butyl, sek. Butyl, Cyclohexyl, Adamantyl, Norbornyl oder Phenyl ist.

4. Ein Verfahren nach irgendeinem der Ansprüche 1 bis 3, wobei R ein geradkettiges oder verzweigtes Alkyl mit 1 bis

4 Kohlenstoffatomen, Phenylethyl, Beta-Cyanoethyl, Morpholino, Piperidino, Thiomorpholino, Beta-Nitroethyl, Beta-niedrigalkylsubstituiertes Sulfonyl oder beta-phenylsubstituiertes Ethyl, ein niederes alkylsubstituiertes oder halosubstituiertes Phenyl oder Benzyl ist.

5. Ein Verfahren nach irgendeinem der Ansprüche 1 bis 4, wobei das Blockierungsmittel ein salz ist, welches durch die folgende Formel definiert ist:



wobei X^+ Amonium, ein niederes Alkylamonium, Pyridin, Lutidin, Cyclohexylamonium oder ein Metallsalzkation ist.

6. Ein Verfahren nach Anspruch wobei, X^+ ein Triethylamonium, Diisopropylamonium, Tetrabutylamonium, Diisopropylethylamonium, Pyridin, Lutidin oder Cyclohexylamonium ist.

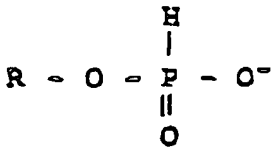
7. Ein Verfahren nach einem der Ansprüche 1 bis 6, wobei das sterisch gehinderte Salzchlorid und das Blockiermittel in äquimolaren Anteilen vorhanden sind.

8. Ein Verfahren zum Synthetisieren eines Oligonucleotids, einer vorbestimmten Sequenz auf einem festen Träger, wobei das Verfahren die folgenden Stufen aufweist:

(a) Entfernen der Schutzgruppe eines 5'-geschützten Oligonucleotids, welches an dem festen Träger befestigt ist, um ein ungeschütztes Oligonucleotid zu bilden;
(b) Reagieren eines 5'-geschützten Nucleotidmonomers mit dem ungeschützten Oligonucleotid, um entweder ein 5'-geschütztes Oligonucleotid oder eine Fehlsequenz zu bilden, wobei die Fehlsequenz eine 5'-Hydroxylgruppe aufweist;
(c) Blockieren der Fehlsequenz durch Reaktion mit einem Blockiermittel mit dem 5'-Hydroxylende der Fehlsequenz durch ein Verfahren nach irgendeinem der Ansprüche 1 bis 7; und
(d) Wiederholen der Stufen (a), (b) und (c) bis das Oligonucleotid der vorbestimmten Sequenz erhalten wird.

Revendications

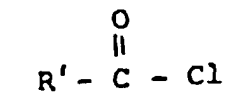
1. Procédé d'addition de chapeau à des séquences de rupture en synthèse oligonucléotidique en phase solide, comprenant la condensation d'un agent d'addition de chapeau avec un hydroxyle d'une séquence de rupture, l'agent d'addition de chapeau étant défini par la formule :



dans laquelle R représente alkyle inférieur, alkyle inférieur substitué par un substituant attracteur d'électrons, aryle substitué par alkyle inférieur ou substitué par halogène, ou un hétérocycle contenant de l'azote, de l'oxygène ou du soufre, ayant de 5 à 8 atomes de carbone.

2. Procédé selon la revendication 1, dans lequel l'agent d'addition de chapeau est condensé avec l'hydroxyle en présence d'un chlorure d'acide stériquement encombré.

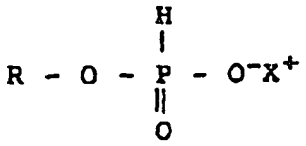
3. Procédé selon la revendication 2, dans lequel R est un alkyle à chaîne droite, ramifié ou cyclique, ayant de 1 à 6 atomes de carbone, morpholinyle, thiomorpholinyle, pipéridinyle, pipérazinyle ou un éthyle substitué en bêta par un substituant attracteur d'électrons, et dans lequel le chlorure d'acide stériquement encombré est défini par la formule :



dans laquelle R' représente tert.-butyle, sec.-butyle, cyclohexyle, adamantyle, norbornyle ou phényle.

4. Procédé selon l'une des revendications 1 à 3, dans lequel R représente un alkyle à chaîne droite ou ramifié, ayant de 1 à 4 atomes de carbone, phényléthyle, bêta-cyanoéthyle, morpholino, pipéridino, thiomorpholino, bêta-nitréthyle, éthyle substitué en bêta par phényle ou par sulfonyle substitué par alkyle inférieur, phényle ou benzyle substitué par alkyle inférieur ou substitué par halogène.

5. Procédé selon l'une des revendications 1 à 4, dans lequel l'agent d'addition de chapeau est un sel défini par la formule :



dans laquelle X^+ représente ammonium, alkyl inférieur-ammonium, pyridinium, lutidinium, cyclohexylammonium ou un cation de sel métallique.

6. Procédé selon la revendication 5, dans lequel X^+ représente triéthylammonium, diisopropylammonium, tétrabutylammonium, diiaopropyléthylammonium, pyridinium, lutidinium ou cyclohexylammonium.

7. Procédé selon l'une des revendications 1 à 6, dans lequel le chlorure d'acide stériquement encombré et l'agent d'addition de chapeau sont présents en quantités équimolaires.

8. Procédé de synthèse d'un oligonucléotide d'une séquence prédéterminée sur un support solide, le procédé comprenant les étapes consistant à :

(a) déproteger un oligonucléotide protégé en 5', fixé au support solide, afin de former un oligonuclèotide déprotégé ;

(b) faire réagir un monomére nucléotidique protégé en 5' avec l'oligonucleotide déprotégé, afin de former soit un oligonucléotide protégé en 5', soit une séquence de rupture, la séquence de rupture ayant un hydroxyle en 5' ;

(c) additionner un chapeau à la séquence de rupture par réaction d'un agent d'addition de chapeau avec l'hydroxyle en 5' de la séquence de rupture par un procédé tel que défini à l'une des revendications 1 à 7 ; et

(d) répeter les étapes (a), (b) et (c) jusqu'à ce que l'oligonucléotide de la séquence prédéterminée soit obtenu.

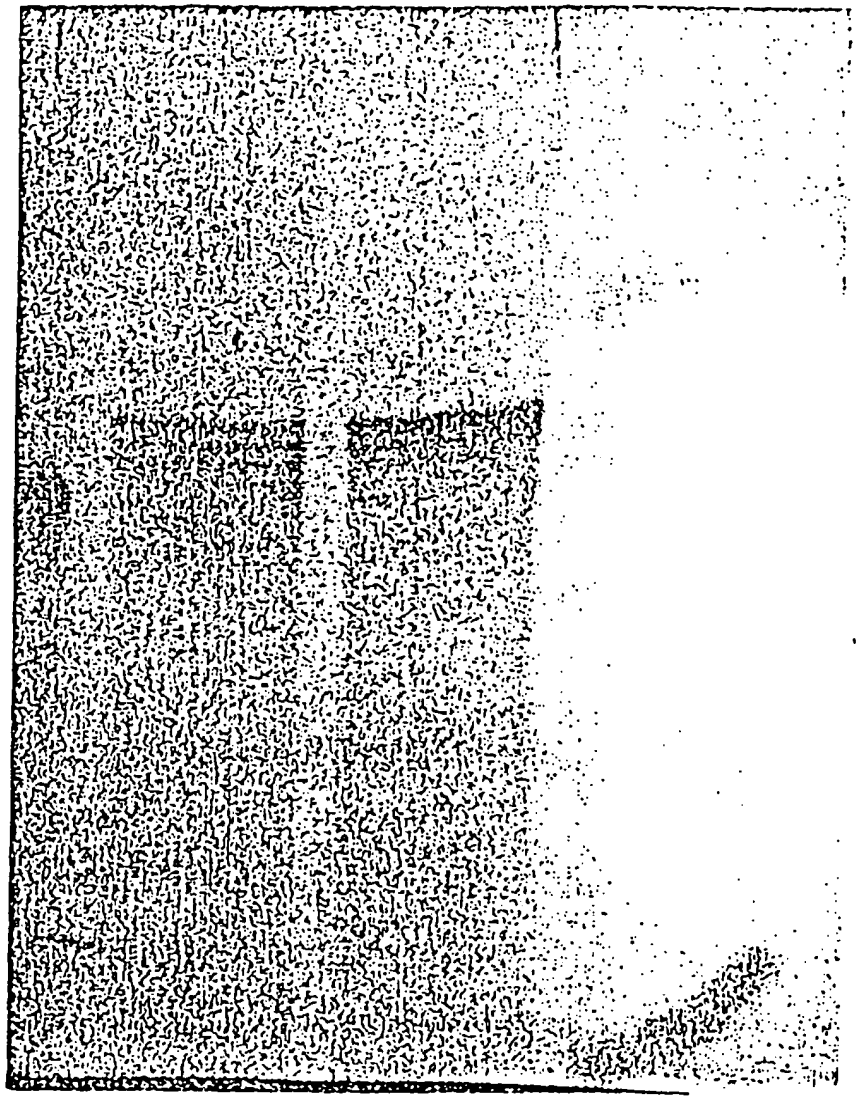


FIGURE 1